THE USE OF RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS AS A SIMPLE TOOL TO DISTINGUISH BETWEEN SALMONELLA ISOLATES IN AN EPIDEMIOLOGIC RESEARCH

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In the Netherlands *Salmonella* is, together with *Campylobacter*, one of the most important causes of bacterial foodborne infections. Although poultry is recognised as the major source of salmonellosis in man, pork also contributes to the human cases of salmonellosis (Van de Giessen, 1996).

A part of the infected pork is due to pigs that have become infected in the last part of their lifes (transportation to the slaughterhouse, waiting in lairage) or during slaughter (Berends *et al.*, 1996). Possible causes of this infection are contaminated transport equipment, mixing of groups during transport and/or lairage, resecretion of *Salmonella* by persistently infected pigs and cross contamination during slaughter by slaughter personnel and equipment.

Aim of this study was to compare RAPD-typing and traditional serotyping of *Salmonella*. The outcome can be useful in the elucidation of the epidemiology of *Salmonella* in this part of the pork production chain.

Therefore identification of different *Salmonella* strains is essential. Since typing with traditional serological methods is time and money consuming, it was decided to use random amplification of polymorphic DNA (RAPD) to distinguish between isolates. The followed procedure was mainly based on the method of van Lith and Aarts (1994).

MATERIALS AND METHODS

Bacterial isolates. In total 392 *Salmonella* isolates were analysed. The isolates were obtained from the lairage of a slaughterhouse during presence of pigs. The samples were taken with a swab (sampling a surface of 50 x 50 cm) and as water samples (100 ml). Salmonellae were isolated by standard procedures (ISO 6579).

Preparation of DNA. The *Salmonella* isolates were grown overnight by 37°C in brain heart infusion broth. One ml of the bacterial culture was centrifuged for 10 minutes at 14000 rpm in an Eppendorf centrifuge. The pellet was washed in 900 µl of a physiological saline solution, centrifuged again 10 minutes at 14000 rpm and then resuspended in 500 µl milliQ water. This suspension was heated for 10 minutes in a thermo-block at 100°C and then cooled by ice, whereafter it was centrifuged for 5 minutes at 10000 rpm. The supernatant fluid was diluted 20 times and used for the RAPD reaction.

RAPD reaction. The primers ERIC1 (3'-CACTTAGGGGTCCTCGAATGTA-5') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') were synthesized by Perkin Elmer. Reaction conditions were standardized by using Ready-to-go-beads (Pharmacia Biotech), containing

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the right amounts of dNTP, Taqpolymerase, Stoffel fragment, BSA and buffer (MgCl2, KCl and Tris). For each reaction 20 µl reaction mix, containing 1 µl ERIC1, 1 µl ERIC2 and 18 µl H2O was added to the beads, together with 5 µl of the diluted DNA suspension. The RAPD reaction mix was overlaid with mineral oil and centrifuged a few seconds to ensure separation of the phases.

Amplification was performed in a Perkin Elmer thermal cycler 480 as follows: one cycle of 7 min at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at 36°C and 4 min at 72°C. The cycling ended with one cycle of 10 min at 72°C. The reaction products were stored at 5°C until electroforesis.

DNA analysis. After RAPD 5 µl loading buffer (containing glycerol, Tris/HCl, EDTA and bromophenol blue) was added to the 25 µl reaction mix. Five µl of this portion was loaded on a polyacrylamide gel (48S ExelGel, Pharmacia Biotech) for electroforesis. On each gel also two control strains (*S. typhimurium* and *S. livingstone*) and 3 markers were loaded routinely.

The DNA fragments were coloured by silver staining (DNA Silver Staining kit, Pharmacia Biotech). After drying the gels were covered with a plastic layer.

Serotyping. From each RAPD type and every gel a representative number of isolates (100 in total) was serotyped at the European *Salmonella* reference centre RIVM at Bilthoven, the Netherlands.

RESULTS

The profiles on the gels showed bright and clear bands, which could be easily judged by eye.

The 392 isolates could be divided in 11 serotypes and 21 RAPD types (see table 1). Every serotype had its own specific RAPD type(s), except for *S. bredeney* (RAPD type B-J) which had the same RAPD profile as some *S. typhimurium*. Within the *S. typhimurium* serotype, RAPD types did not match with the phage types. The reference strains *S. typhimurium* and *S. livingstone* showed the same profile on every gel (10 gels so far).

DISCUSSION AND CONCLUSIONS

The profiles of the reference strains were consistently comparable, so that it is allowed to match all the profiles at the different gels together, thus improving the use of this method substantially. Probably the standardisation of the procedures (use of Ready-to-go-beads) and the use of a polyacrylamide gel count for the improvement of the reproducibility.

The used RAPD-technique seems a powerful method for typing *Salmonella*. Only a small amount of DNA is needed, which makes it possible to extract DNA by heating instead of chloroform/phenol extraction. Instead of PCR, which technique requires knowledge of the DNA sequence of the target organism, RAPD has the advantage of using primers with arbitrary oligonucleotide sequence, which makes this method general usable.

Compared to the results of Van Lith and Aarts (1994) the profiles contained more bands which was probably due to the use of a polyacrylamide gel instead of an agarose gel. This higher discriminatory effect may have been the reason for the observation that in this experiment a number of serotypes contained more than one RAPD type. Van Lith and Aarts (1994) observed only one RAPD type per serotype.

Since every serotype has its own RAPD type(s) (except for *S. bredeney*) it will be possible

to use RAPD as a simple alternative for serotyping of *Salmonella*, which can save a lot of time and money.

Table 1. Serotypes and corresponding RAPD types of *Salmonella* isolates originating from the lairage of a pig slaughterhouse

Serotype	RAPD-types	Number(per type)
S. infantis	C-A	29
S. livingstone	C-B	2
	C-E	13
S. bovismorbificans	C-C	9
S. mbandaka	C-F	7
S. give	E-A	18
S. london	E-B	12
S. typhimurium	B-A	88
	B-D	1
	B-E	1
	B-H	1
	B-J	5
	B-K	3
S. brandenburg	B-B	1
	B-G	2
	B-I	17
S. derby	B-F	1
S. panama	D-A	169
	D-E	5
	D-F	1
	D-G	5
S. bredeney	B-J	2

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